National Research Council Canada
Conseil national de recherches Canada

EXTRACTIVE FERMENTATION USING FLUORINATED HYDROCARBONS

BY
ECO-RESEARCH
(CANADA) INC.

DIVISION OF ENERGY / DIVISION DE L'ÉNERGIE

Canada

NRCC 23979

-3208/

This report summarizes work carried out under contract to the Division of Energy, National Research Council of Canada. It has been published in accordance with the policy of the National Research Council of Canada of making information on energy research and development available to all interested groups. Publication by the National Research Council of Canada does not signify that the contents necessarily reflect the views or policies of the National Research Council of Canada, nor does mention of trade names or commercial products constitute endorsement or recommendation for use. Furthermore, no warranty or representation is made with respect to the accuracy or completeness of the information contained in this report. This report is reproduced here from camera-ready copy prepared by the contractor. The abstract was translated by the National Research Council of Canada.

Le présent rapport constitue un résumé des travaux effectués à contrat par la Division de l'énergie, Conseil national de recherches du Canada (CNRC). Sa publication est conforme à la politique du CNRC qui est de diffuser l'information disponible sur la recherche et le développement énergétiques auprès de tous les groupes intéressés. Son contenu n'exprime pas nécessairement les opinions ou politiques du Conseil national de recherches du Canada et la mention de marques ou produits commerciaux ne doit pas être considérée comme une approbation ou une recommandation d'utilisation. De plus, aucune garantie ni représentation ne peut être faite quant à l'exactitude ou à l'intégralité du contenu du rapport, qui a été reproduit à partir d'une copie prête à l'impression préparée par le sous-traitant. Le résumé a été traduit par le Conseil national de recherches du Canada.

Copyright 1984 by
National Research Council of Canada

Permission is granted to quote short excerpts and to reproduce figures and tables from this report, provided that the source of such material is fully acknowledged.

Copies of this and other publications of the Division of Energy may be obtained by mailing the appropriate remittance (a Bank, Express, or Post Office Money Order, or a cheque made payable to the Receiver General for Canada, credit NRC) to

Publication Sales and Distribution Office
National Research Council of Canada
Ottawa, Ontario
K1A 0R6

Price: $2.50

Copyright 1984 par
Conseil national de recherches du Canada

De courtes citations et la reproduction de figures et tableaux du présent rapport sont autorisées sous réserve que la source en soit clairement mentionée.

Des exemplaires du rapport et d'autres publications de la Division de l'énergie peuvent être obtenus par courrier sur le paiement d'avance des droits requis (règlement par mandat de banque, mandat-exprès, mandat-poste ou chèque payables au Receveur général du Canada, crédit du CNRC) à l'adresse suivante:

Bureau de vente et diffusion des publications
Conseil national de recherches du Canada
Ottawa (Ontario)
K1A 0R6

Prix unitaire: 2,50 $

EXTRACTIVE FERMENTATION USING FLUORINATED HYDROCARBONS

CONTRACT NO. 055X.31926-3-0049

FINAL REPORT

prepared by

Eco-Research (Canada) Inc.

April, 1984

TABLE OF CONTENTS

		Page
1.	SUMMARY/RÉSUMÉ	v
2.	INTRODUCTION	1
3.	Materials and Methods	4
	3.1 Organisms employed	4
	3.2 Extractants selected	4
	3.3 Distribution coefficient determination	5
	3.4 Culture conditions	6
4.	Results	8
	4.1 Distribution coefficients	8
	4.2 Toxicity of Extractants	8
	4.3 Acclimatization of yeast	9
	4.4 Recovery of ethanol	10
5.	Discussion	12
	5.1 Extraction efficiencies	12
	5.2 Toxicity and acclimatization	14
	5.3 Process selection	15
	5.4 Viability of the process	17
6.	Conclusions	20
7.	References	21

LIST OF FIGURES

1. *Saccharomyces cerevisiae* batch fermentation 22

LIST OF TABLES

Page

1. Distribution coefficients and separation factors of ethanol in water and organic solvents 23

2. Effect of various extractants on the growth and alcohol formation by S. cerevisiae 24

3. Effect of various extractants on the growth and alcohol formation by Z. mobilis 26

4. Comparison of the two continuous flow fermenters . . 27

5. The volume recovered at various temperatures during the distillation of 10% alcohol solution in Fluorocarbon II 28

6. Effect of Fluorocarbon II on growth and alcohol fermentation 29

SUMMARY

A range of fluorinated hydrocarbons have been evaluated as solvents in extractive fermentation. Critical factors studied have included the efficiency of ethanol extraction from aqueous solution, and the toxicity of solvents to the organisms employed.

In general, the distribution coefficients of ethanol between the solvent and water are rather low, but the selectivities of the solvents for extracting ethanol over water are good. The toxicities of the solvent to the yeast vary from extremely toxic to non-toxic.

Based on the results obtained, the viability of an extractive fermentation process employing fluorinated hydrocarbons has been evaluated.

RÉSUMÉ

Tout une gamme d'hydrocarbures fluorés ont été évalués en tant que solvants utilisés pour la fermentation extractive. Les facteurs importants étudiés comprenaient l'efficacité de l'extraction de l'éthanol à partir de solutions aqueuses et la toxicité des solvants pour les organismes employés.

En général, les coefficients de distribution de l'éthanol entre le solvant et l'eau sont plutôt faibles, mais les facteurs de sélectivité des solvants utilisés pour extraire l'éthanol dans l'eau sont bons. La toxicité des solvants pour la levure va de très toxique à non toxique.

La viabilité d'un processus de fermentation extractive employant des hydrocarbures fluorés a été évaluée en fonction des résultats obtenus.

2. INTRODUCTION

The fermentation production of fuel alcohol can offer an attractive energy source from renewable or waste starch and cellulose. However, the process suffers from a failing common to all fermentations: the chemical is produced as a dilute aqueous solution. For a low value product such as ethanol, the cost of concentration to 99.5% fuel ethanol can seriously prejudice the economic viablity of production. An additional problem increasing the costs of fermentation is that of product inhibition, with increasing ethanol levels inhibiting the fermentation (this becomes important at about 5% ethanol for Saccharomyces cerevisiae and at lower levels for other organisms).

A number of schemes have been proposed to overcome these limitations: modified distillation processes to minimize energy requirements (1); reverse osmosis ethanol concentration (2); vacuum fermentation to remove ethanol as it is formed (3); employing hexane to azeotropically distil the ethanol (4); 'plug-flow' fermentations so that inhibition only affects rates in the later reactor stages (5); and extractive fermentation (6-7).

Extractive fermentation has the potential both to minimize product inhibition by removing ethanol as it is formed, and by utilization of a suitable solvent, to minimize ethanol concentration energy requirements.

Recently Hernandez-Mena et al (6) and Minier and Goma (7) have studied extractive fermentation with two solvent systems, while Roddy (8) and Paciorek et al (9) have evaluated a range of solvents for their ability to extract ethanol from water. The results of Minier and Goma are impressive: using dodecanol as extractant to maintain ethanol below inhibiting levels, they were able to obtain from continuous fermentation ethanol productivities 4 to 5 times higher than without extraction. Complete utilization of a 409 g/L ferment syrup was achieved compared to only partial utilization of 200 g/L without extraction.

However dodecanol is a solid at room temperature which increases handling problems, and membrane technology must be developed for ethanol/dodecanol separation. Other solvents employed, dibutylphthalate (6) and long chain fluorinated acids (9) also would require technology development to separate the extracted ethanol. In

addition, none of these solvents possesses the extractant characteristics required for high efficiency extraction as is described below.

An ideal solvent for extractive fermentation of ethanol should have the following chracteristics:

i) To obtain azeotropic (95%) ethanol from a 5% ethanol solution by solvent extraction, the separation factor (S.F.) must be in the order of 300-400. The separation factor is a ratio of distribution coefficients, i.e.

$$S.F. = \frac{DEthanol}{Dwater} = \frac{[C_2H_5OH]org}{[C_2H_5OH]aq} \quad \frac{[H_2O]org}{[H_2O]aq}$$

ii) D Ethanol should be as large as possible so that too great an inventory of solvent is not required. On the other hand the solubility of the solvent in water must be as low as possible or unacceptable solvent losses will result.

iii) The solvent must not be toxic or inhibiting to the organisms employed at the solvent levels employed in the fermenter. This level will depend on the nature of the process selected.

iv) The separation of ethanol and solvent should require minimal energy and preferably not necessitate the development of additional technology.

A suitably selected fluorinated hydrocarbon could encompass all these characteristics: acidic hydrogen atoms (found on carbon atoms bonded to halides in the fluorinated hydrocarbon) will enhance the extraction of ethanol from water. The boiling point of the extractant can be chosen so that ethanol separation from the extractant by distillation requires minimal energy. The selection of fluorinated hydrocarbons with potentially suitable properties has increased recently because of their development for heat pump applications.

This project has been undertaken to assess the technical and economic viability of employing fluorinated hydrocarbons for extractive fermentation.

The objectives of the project are:

i) To evaluate a range of fluorinated hydrocarbons for use in extractive ethanol fermentation. Critical factors studied are any toxic effects on the organisms employed, and the efficiency of ethanol extraction from aqueous solution. No fermentation process development is included in the project.

ii) To evaluate the technical and economic viability of a process employing extractive fermentation with fluorinated hydrocarbons.

3. Material and Methods

3.1 Organisms employed

The selection of cultures was made in consultation with Ms. Lyse Morriset and Dr. Bert Van den Berg of NRC. *Saccharomyces cerevisiae* was selected as the main test culture due to its almost universal use in commercial ethanol production. Two additional cultures of potential commercial interest, the ethanol producing bacterium *Zymomonas mobilis* and the butanol/acetone producer *Clostridium acetobutylicium* were initially selected for comparison purpose. However, only the former *Z. mobilis* was used in this study. Bacterial culture was obtained from the NRC culture collection, and the *S. cerevisiae* from Sigma Chemicals and the NRC culture collection.

3.2 Extractants selected

The various fluorinated hydrocarbons used in this study are listed below. This list also includes 1-dodecanol which is not a fluorinated compound, but which was included in the project to allow comparison of results with other workers.

		Source	Boiling Point °C
1) CCl_3F	Fluorocarbon 11	Air Products	24
2) $CF_3CH_2OCF_2CF_2H$	Fluorinated diethyl-ether	ICI Mond Division	57
3) $CF_3CHClBr$	1-Bromo-1-Chloro-2,2,2-Trifluoroethane	Columbia Organic Chemical	50
4) $CHClFCF_2OCH_3$	2-Chloro-1,1,2-Tri-fluoro ethyl methyl ether	Columbia Organic Chemicals	71
5) $CHClFCClF_2$	Arcton 123A	ICI Mond Division	28
6) $CHF_2CF_2OCH_3$	1,1,2,2-Tetra fluoro ethyl methyl ether	ICI Mond Division	29
7) C_6F_{14}	Perfluorohexane	Columbia Organic Chemicals	59-60
8) $CH_3CH_2CH_2CH_2F$	1-fluoro butane	Columbia Organic Chemicals	32
9) $CH_3(CH_2)_{11}OH$	1-Dodecanol	Aldrich	259 (MP24)

These compounds were used for both extraction experiments and to observe their toxicity to the microorganisms being used.

3.3 Determination of Distribution Coefficients

Initially the extraction of alcohol from aqueous solution by various fluorinated hydrocarbons was carried out at room temperature. The two phases were agitated in a separatory funnel for two minutes and the alcohol content analyzed after the two phases have separated.

To avoid the possibility of loss of the volatile compounds during vigorous shaking at higher temperature the procedure was later modified and the agitation and separation was carried out in a cold room at 10°C. The glassware and the chemicals were also cooled to this temperature prior to their use. In each case 30 ml of 1 molar ethanol solution in water was added to 20 ml of the test solvent in a 250 ml conical flask. The mouth of the flask was tightly closed with rubber stoppers wrapped in aluminum foil. The flasks were shaken for one hour in a rotatory shaker at 350 rpm. The liquid after shaking was transferred to a separatory funnel and allowed to separate overnight. The experiment with 1-dodecanol was carried out at 25 and 35°C because dodecanol is a solid at lower temperatures. In all cases the two layers of liquid, aqueous and organic, were analyzed for alcohol. In addition the aqueous portion was also analyzed for the solvent and the organic phase for water.

A Water's Associates HPLC with differential refractometer detector was employed to determine alcohol in the aqueous phase. Ethanol in the organic phase as well as the solvents in the aqueous phase were determined by gas chromatography. Water in the organic phase was determined by Karl Fisher method.

In experiments involving fermentation, both alchohol and glucose in the fermentation broth were determined by HPLC using a Sugar Pak I column.

Distribution coefficients of both alcohol and water as well as the separation factors (S.F.) were calculated according to method used by Roddy (8).

$$\text{DEthanol} = \frac{[C_2H_5OH]org}{[C_2H_5OH]aq} \qquad \text{S.F.} = \frac{\text{DEthanol}}{\text{DWater}}$$

$$\text{DWater} = \frac{[HOH]org}{[HOH]aq}$$

3.4 Culture Conditions

The compositions of the media used to grow S. cereivisiae and Z. mobilis are shown below.

For S. cerivisiae		For Z. mobilis	
Glucose (Anhydrous)	100.0 g	Glucose (Anhydrous)	20.0 g
Yeast Extract	8.5 g	Yeast Extract	2.5 g
NH_4Cl	1.32 g	KH_2PO_4	1.0 g
$MgSO_4,7h_2O$	0.11 g	$(NH_4)_2 SO_4$	1.0 g
$CaCl_2$	0.06 g	$MgSO_4, 7H_2O$	0.5
Antifoam (Tween 80)	0.2 ml		
		Tap Water To make	1L
Tap Water To make	1L		

Glucose and minerals in both cases were sterilized separately to avoid caramelization and the two cultures, Saccharomyces and Zymomonas, were propagated in shake flasks at 35°C.

Toxicity tests were done by transferring 25 ml double strength culture medium, 5 ml of seed and the organic solvent in varying amounts into the serum bottles. The required volume of dilution water was then added to the bottles to make the final volume up to 50 ml. The bottles were incubated in a shaker-incubator maintained at 35°C and harvested after 24-48 hours.

Biomass in the case of yeast was obtained by fltering the broth through a Whatman GF/C glass microfiber filter. Filtration in the case of Z. mobilis was done with millipore (0.45μ pore size) filters. The filters were dried overnight at 110°C and weighed to obtain the biomass. The filtrate was analyzed to obtain the amount of alcohol produced and glucose utilized during fermentation. Typical changes with time in biomass and ethanol level during the growth of S. cerevisiae are shown in Figure 1.

S. cerevisiae was also grown in two separate fermenters which were operated in the continuous mode. The parameters for the operation of these fermenters were

Volume	2L
Flow Rate	1L/day
Hydraulic Retention Time	2 days
Temperature	35°C
Agitation	100 rpm
Aeration	None (anaerobic)

The media used in these fermenters were the same as described before for S. cerevisiae. 5.0g/L of Fluorocarbon 11 (CCl_3F) per day was also added to one of the fermenters to acclimatize the yeast to this compound. Seeds from this fermentor were used to do toxicity tests to see if acclimatization helps in minimizing the growth and toxicity effect.

4. Results

4.1 Distribution Coefficients

The results of the distribution experiment carried out with 8 fluorinated compounds at 10°C and with 1-Dodecanol at 25 and 35°C are shown in Table 1. These distribution values are low but are in the same range as reported by Roddy (8) and other workers where comparison is possible. These experiments as mentioned earlier were carried out at 10°C to avoid the loss of volatile compounds and could have resulted in lower distribution coefficient values. Roddy in his work with a large number of organic compounds had observed that better extraction could be achieved with an increase in the temperature. The separation factor on the other hand appears to be independent of temperature.

The 1-Fluorobutane sample was obtained from Columbia Organic Chemical Co. of Camden, South Carolina in U.S.A. and was delivered in a metal pressure bottle. On removal from this container it was found to be of pale yellow colour with appreciable amount of solid particles. Filtered samples were used in the experiment but it is suspected that the impurities in it could have caused the analytic problem which recurred when injected into gas chromatography column. A large number of peaks were observed which overlapped the peaks observed for alcohol. It has since been learned that fluorobutane may be susceptible to hydrolysis to butanol (private communication R. Powell, I.C.I., Mond Division). The DEthanol of 4.0 observed strengthens this possibility as the DEthanol for butanol is in this range.

4.2 Toxicity of the extractants

4.2.1 S. cerevisiae

The effects of increasing quantities of various organic solvents on the growth and alcohol production by yeast growing in serum bottles are shown in Table 2.

Among the nine compounds tested, $CHClFCF_2OCH_3$, $CHClFCClF_2$ (Arcton 123A) and $CH_3(CH_2)_2CH_2F$ are the most toxic to the growth of yeast with inhibition approaching 95% at a 0.5% concentration of organic solvent in the fermentation broth. 90% growth inhibition was also observed in the case of $CF_3CHClBr$ and $CHF_2CF_2OCH_3$ but only at a concentration above 1%. Inhibition in the case of CCl_3F reached only 50-60% even when its concentration in the broth went up to 10%. In all cases mentioned so far the alcohol production and glucose utilization was virtually eliminated at solvent concentrations as low as 0.5%.

Experiments for C_6F_{14} (perfluorohexane) could not be repeated due to the unavailability of the chemical, but the results of preliminary experiments showed that it is not toxic to growth even at 10% level.

$CF_3CH_2OCF_2CF_2H$ appears to be another compound with relatively low toxic effect on growth and no effect on alcohol formation by yeast.

1-Dodecanol was also examined as a control chemical and appears to be significantly toxic at even 0.2%, reducing yeast growth by over 60%, and severely inhibiting ethanol production. These reults which were very reproducible when repeated, disagree with those obtained by Minier and Goma (7), who found little toxicity. Murphy et al (10) on the other hand, state that the higher alcohols are toxic in saturated aqueous solutions (less than 0.1%). These differences may perhaps be due to the fact that a different yeast strain was employed by Minier and Goma, or because the fermentation conditions differed (Minier and Goma employed an immobilized yeast in a packed bed.)

4.2.2 Z. mobilis

The toxicity of only two fluorinated compounds were tested with this organism (Table 3). Unfortunately, no growth could be observed even in the control, and so it was decided to concentrate further work on S. cerevisiae.

4.3 Acclimatization of Yeast to CCl_3F

As described earlier S. cerevisiae was grown in two fermenters, one of which received 5g of CCl_3F (2.5g/L) each day to acclimatize the yeast to the presence of Fluorocarbon 11. The fluorocarbon generally settled into

the bottom of the fermenter. However agitation of the fermenter broth at 100 rpm accomplished some mixing. There is a possibility that Fluorocarbon 11 may have volatalized at the operational temperature of 35°C and escaped from the system. However, due to the daily addition of this compound to the fermenter, the yeast cells must have experienced some exposure to the toxic effect of the fluorocarbon. The biomass, glucose and alcohol concentrations in the fermenter were determined on a regular basis and the results are presented in Table 4. The fermenter with Fluorcarbon 11 had substantial growth but biomass concentration was consistently lower than that of control. Glucose consumption and alcohol production appears to be also marginally affected by the presence of Fluorocarbon 11.

A toxicity test with Fluorocarbon 11 was also carried out with yeast inoculum obtained from the following three different sources.

1) From the fresh culture being propagated in flasks

2) From a fermenter operating under continuous feeding mode.

3) Same as No. 2 but provided with additional daily dose of 5g CCl_3F.

The results of this study are shown in Table 6 and it appears that in every case the growth of yeast is drastically inhibited in the presence of Fluorocarnon 11. The yeast obtained from fermenters appear to show even higher susceptability with growth inhibition exceeding 90% at Fluorocarbon concentration of 1%. However, the inocculum obtained from fermenter #2 appears to have gained some resistance to low concentrations (0.5%) of Fluorocarbon 11 as far as alcohol production is concerned. Under these conditions there was some alcohol formation and corresponding glucose utilization.

4.4 Recovery of ethanol

One of the perceived advantages in the use of low boiling point solvents to extract alcohol from fermentation broth is the possibility of energy savings and ease in the final separation of alcohol from these solvents. To study the distillation characteristics of a mixture of alcohol in

Fluorocarbon 11, a 90% solution of alcohol in water was prepared. A 10% solution of this aqueous mixture in Fluorocarbon 11 (CCl_3F) was then made and distilled under controlled conditions. The volume of the distillate obtained at various temperatures is shown in Table 5. Because of the high volatility of the Fluorocarbon 11 the condenser was packed with dry ice but despite this precaution 23.5 ml of freon was lost during distillation.

A system to trap the Fluorocarbon 11 can be relatively easily constructed and distillation losses can be minimized without much difficulty. From these data it is clear that all the fluorocarbon can be removed from the mixture below 30°C. The energy requirement for the separation of alcohol under these condition will be much lower than that from a purely aqueous mixture (see section 5.4).

5. DISCUSSION

5.1 Extraction Efficiencies

Three factors are most useful in assessing the extraction efficiency of a solvent: DEthanol, the distribution coefficient of ethanol between the solvent and water; S.F., the separation factor, which is a measure of the solvents selectivity for ethanol over water; and the solubility of the solvent in water. For the nine solvents evaluated in this project these factors are listed in Table 1. The ideal solvent should have D Ethanol as high as possible to minimize the volume of solvent required. Even more important S.F. must be high or the separation of ethanols and water will not have been accomplished by the extraction. An S.F. of >300 is necessary to produce azeotropic (95%) ethanol from a 5% aqueous solution.

Finally the solubility of the solvent in water must be extremely low to avoid unacceptable solvent loss, although this might also be minimized by water recycling.

Obviously the fluorinated hydrocarbons do not possess these ideal properties. However because water and ethanol have very similar solubility and miscibility characteristics, they are extremely difficult to separate, and no chemical, regardless of price, has been identified with ideal extractant properties. In fact the fluorinated hydrocarbons compare quite favourably with some other classes of solvent investigated (9), as summarized below:

Solvent	D Ethanol	S.F.	Solvent Water Solubility (%)
Fluorinated Hydrocarbons	0.01-0.06	10-200	0.01-0.20
Higher Alcohols	0.2 -0.8	10-40	0.01-0.06
Esters	0.2 -0.3	10-20	0.2 -1.0
Ketones	0.1 -0.2	10-20	0.2 -2.0
Amines	0.01-0.4	0.1-10	0.1 -2.0
Phosphates	0.2 -0.6	10-20	0.05-0.5
Alkanes	0.005-0.008	100-300	0.001-0.06
Aromatics	0.02-0.1	90-120	0.01-0.2
Long Chain Fluorinated Acids	0.5 -1.5	10-30	-

In all solvent classes as DEthanol increases, S.F. decreases, and this trend is observed amongst the fluorinated hydrocarbons also. The fluorinated hydrocarbons containing acidic protons exhibited the higher D Ethanol values, and there is some indication that the fluoro-cloro-hydrocarbons provide a better combination of DEthanol and S.F. than the purely fluorinated materials. Only a limited number of suitable fluorinated hydrocarbons were available for testing, but it appears unlikely that any other chemical in this class will exhibit a major improvement in extraction efficiency.

The anomalously high value of DEthanol determined for fluorobutane (4.0) is almost certainly due to its hydrolysis to butanol, and so this chemical cannot be considered as a suitable extractant.

The effect of varying S.F. values can best be appreciated by looking at some examples:

	S.F.	Ethanol concentration extracted from 5% aqueous ethanol
CCl_3F	200	90%
$CHClF-CClF_2$	58	72%

In fact CCl_3F and $CHClF-CClF_2$ are probably the two most promising extractants. The S.F. for $CHClF-ClIF_2$ is lower than for CCl_3F but its DEthanol is six times higher.

Two approaches might be employed for improving the extraction properties of the fluorinated hydrocarbons:

- a mixed solvent system could be employed with the goal of achieving properties better than either solvent component.
- in the aqueous phase, the activity of ethanol relative to water can be increased by the addition of a soluble salt. Murphy et al (10) have found that KCl is the most promising (least toxic) salt, but of course water recycling must be practiced if KCl is employed for enhancing the extraction process.

5.2 Toxicity and Acclimatization

The fluorinated hydrocarbons evaluated exhibit a wide range of toxicities to S. cerevisiae: several are toxic at 0.1% addition, while $CF_3CH_2OCF_2CF_2H$ and C_6F_{14} cause little inhibition even at 5% or higher additions.

For Fluorocarbon 11 (CCl_3F), which has a relatively high toxicity, the preliminary continuous fermentation experiments indicated that some acclimatization of the yeast can be achieved. It is probable that with suitable choice of fluorinated hydrocarbons, and a more extensive acclimatization program, a system non-toxic to the yeast at any level can be developed.

Other potential solvents again show a wide range of toxic behaviour : 1-dodecanol was found to be toxic in this study, but Minier and G. Goma fourd it non-toxic (7). They found the lower alcohols extremely toxic. Alkanes show no toxicity, but aromatics and phosphates are moderately toxic.(10).

5.3 Process Selection

Three solvent properties will dominate in the selection of an extractive fermentation process; these are extraction efficiency, toxicity and volatility:

- Extraction efficiency. The higher D Ethanol, the smaller the solvent inventory required. Unless the solubility of the solvent in water is extremely low, water recycling will be an essential element of the process. S.F. must be > 300 if azeotropic alcohol is to be produced from fermentation broths. Lower S.F.'s may necessitate the addition of a further ethanol concentration stage.

- Toxicity. Only solvents which are non-toxic or exhibit extremely low toxicity can be employed directly in the fermenter vessel. For more toxic solvents, aqueous ethanol - solvent contacting must be performed in a vessel separate from the fermenter. For solvents toxic at their solubility level in water an extractive fermentation process may not be viable.

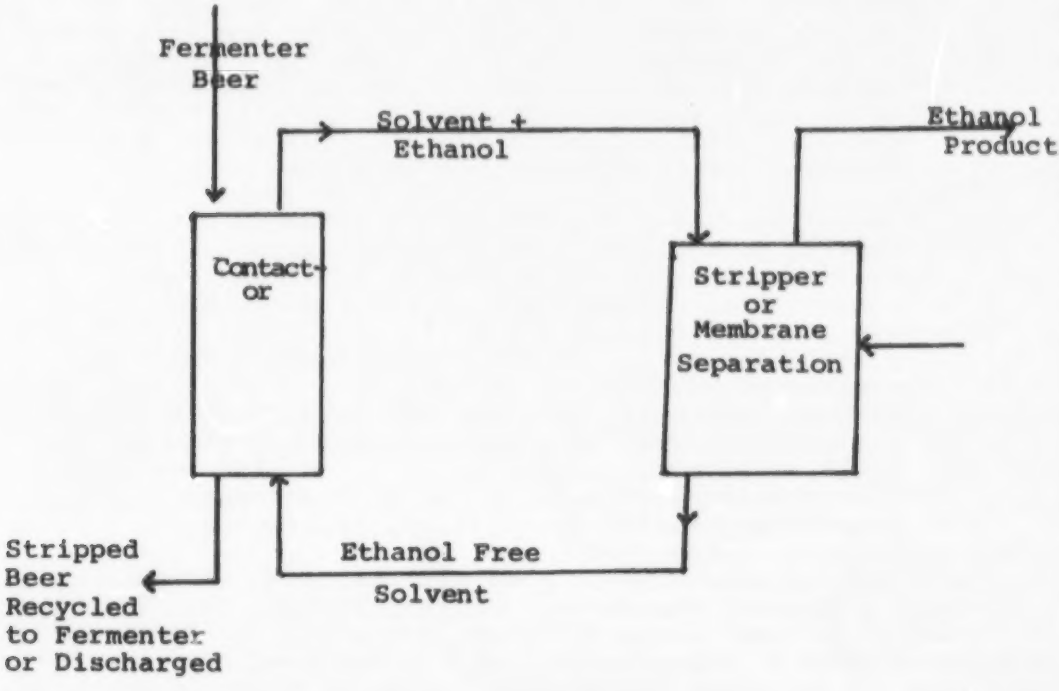
- Volatility. For low volatility solvents with very high boiling points a distillation separation of the solvent and ethanol will provide little advantage over conventional distillation concentration, although avoidance of product inhibition may still justify the utilization of extractive fermentation. Membrane separation methods may eventually provide a lower energy ethanol concentration route for involatile solvents. For solvents with very high volatility, such as the fluorinated hydrocarbons employed in this project, distillation separation might provide energy savings over ethanol-water distillation separation.

Consideration of these factors is used below to select the most advantageous process for extractive fermentation with fluorinated hydrocarbons. For these solvents, highest S.F. found was 200, and the highest D Ethanol was 0.06. These values were for two different fluorinated hydrocarbons, but by the use of a mixed solvent system, or by salt additions (see Section 5.1.) it should be possible to achieve these two values simultaneously. The other typical properties which can be expected are, solvent water solubility of 0.1%, boiling point of 30°C, and toxicity to yeast at above 5% in the fermentation stage : only 5% of the solvent can be tolerated by the yeast, and so the extraction process

would most effectively be carried out in a separate vessel. A packed bed bioreactor containing an immobilised yeast would facilitate this extraction.

Assuming an ethanol concentration of 10% in the fermentation beer, the solvent after mixing will contain about 0.6% ethanol plus 0.06% water, and approximately 16 volumes of solvent will be required to completely extract each volume of fermentation beer. For each volume of ethanol extracted, 9 volumes of water will be processed, and 0.009 volumes of solvent will enter this water phase. Separation of the solvent and ethanol phases might be conducted by distillation at 30°C, or by membrane separation etc.

A schematic diagram of this process is shown below.



This basic process, with either distillation or membrane separation option, will provide the most efficient utilization of fluorinated hydrocarbon extractants. In Section 5.4 the energy requirements and economic viability of this process is assessed.

5.4 Viability of the Process

i) Energy Requirements

For distillation separation of the fluorinated hydrocarbons and ethanol to be an attractive option, it must have lower energy requirements than conventional ethanol-water distillation separation.

An approximate evaluation for concentration of a 10% ethanol fermentation beer is given below:

- Conventional distillation.

  To heat 10% ethanol from 30°C to 78°C and distil azeotropic (95%) ethanol requires about 2800 J/g ethanol.

- Extractive fermentation followed by distillation separation.

  Using the example developed in Section 5.3., the 10% ethanol will be extracted with 16 volumes of solvent yielding a 0.6% ethanol solution. To distil this solution bp. 30°C will require only approximately 160 J/g of solvent (this is a typical heat of vaporization for a fluorinated hydrocarbon). However when this is calculated in terms of ethanol produced, the requirement is

$$160 \times \frac{99.4}{.6} = 26500 \text{J/g ethanol}$$

  Comparing these values it is obvious that distillation separation will not be viable for such a dilute solution of ethanol in fluorinated hydrocarbon. In fact, only solutions containing more than about 6% ethanol in fluorinated hydrocarbon can be distilled with a lower energy requirement than conventional distillation of 10% ethanol. From the extraction results obtained in this project it appears very unlikely that any fluorinated hydrocarbons will be developed that can provide 6% ethanol in solvent from 10% aqueous ethanol, and so the distillation option will not be viable. Other processes with lower energy requirements for ethanol-fluorinated hydrocarbon separation may be developed, such as membrane

separation, but no estimation of efficiency or energy balance can be made. One positive feature of the fluorinated hydrocarbons is that because of the high separation factors achievable, 90% or higher ethanol (10% or less water) can be extracted from dilute beers.

It is worth noting that solvents investigated by other workers will not suffer from the same problems, but are unlikely to provide an energy efficient route for ethanol concentration. For example with dodecanol or the long chain fluorinated acids, the solvent is non-volatile, so ethanol may be distilled from the solvent as in conventional distillation, although if the ethanol solution is more dilute than the fermentation beer the energy requirements will probably make this option unattractive. An additional problem for these solvents is that their low separation factors will result in an ethanol-water mixture of only about 60% ethanol, further complicating the separation process.

ii) The Potential for Increasing Ethanol Productivity

Extraction of the ethanol before it reaches concentrations inhibitory to the organism has the potential to increase ethanol productivity, thereby reducing capital and operating costs. The experiments performed in this project looked only at the toxic effects of solvents employed. However, Minier and Goma (7) have demonstrated the ability to increase ethanol production with 1-dodecanol as an extractant, and it is possible that similar advantages would be achieved using fluorinated hydrocarbons. Unfortunately, for any extractive fermentation process there is an obvious trade-off here: the lower the concentration of ethanol in the fermentation beer, the less product inhibition will occur, but unavoidably also the more ethanol concentration will be necessary.

Because of this the further development of extractive fermentation may depend upon the introduction of an efficient isothermal process (probably membrane based) to separate ethanol and the solvent.

In terms of solvent cost the fluorinated hydrocarbons range from about $1.50/kg for trichloro-fluoromethane, to an upper limit of about $6.50/kg for some of the fluorinated ethers evaluated in this project. By comparison, 1-dodecanol is priced around $1.10/kg, and the long chain fluorinated acids are believed to cost more than $10/kg. As all solvent classes have advantages and disadvantages, the choice of solvent for extractive fermentation will probably also depend upon which type of solvent can be most efficiently membrane separated from ethanol.

6. CONCLUSIONS:

i) Volatile fluorinated hydrocarbons can be employed as solvents to extract ethanol from aqueous solutions. The distribution coefficients of ethanol between the solvent and water, DEthanol is low for all the chemicals tested, but the selectivity of the solvent for water over ethanol, S.F., is higher than most other classes of solvent.

ii) The fluorinated hydrocarbons range from extremely toxic to non-toxic for yeast. There is some evidence that yeast can be acclimatized to higher levels of the solvents.

iii) Extractive fermentation employing fluorinated hydrocarbons will not be viable unless an efficient isothermal process for separating the extracted ethanol-solvent mixture can be developed. In fact this same development will be necessary for extractive fermentation with any solvent chemical investigated to date to be viable.

7. **REFERENCES:**

1. "Technology and economics of fermentation alcohol-an update" C.R. Keir, Enzyme. Microb. Technol 5, 103-114, 1983.

2. "Preliminary studies on reverse osmotic separation of ethyl alcohol present in high concentrations in aqueous solutions". Y. Taketani, B. Kurst, T. Matsuura, and S. Saurirajan, Proceedings Third Bioenergy Symposium 145-150, 1981.

3. "The vacuferm process" A. Ramalingham and R.K. Finn, Biotechnology and Bioengineering 19, 583-9. 1977.

4. "Preliminary evaluation of the low-temperature distillation of alcohol during fermentation", R.K. Finn and R.A. Boyajian in Abstracts 5th Intl. Fermentation Symposium, ed. H. Dellweg p 376. Berlin: Published by Westko, 1976.

5. "Method for carrying out continuous fermentation" L.K.J. Ehnstron, British Patent 1422076 (Jan. 21, 1976).

6. "Demonstration of process feasibility for continuous extraction of alcohols from fermenting systems", R. Hernandez-Mena, J.A. Ribaud and A.E. Humphrey, Proceedings of the 6th Intl. Fermentation Symposium, London, Ontario, 1980.

7. "Ethanol production by extractive fermentation", M. Minier and G. Goma, Biotechnology and Bioengineering, 24, 1565-79, 1982.

8. "Distribution of ethanol-water mixtures to organic liquids", J.W. Roddy. Ind. Eng. Chem. Process Dev. 20, 104-108, 1981.

9. "Extraction of ethanol with fluorocarbons" K.L. Paciorek, D.W. Harris, S.R. Ahmed, and R.H. Kratzer U.S. Department of Energy Report DOE/ID/12316 .....Tl, December 1982.

10. "Water recycling in extractive fermentation" T.K. Murphy, H.W. Blanch and C.R. Wilke. Process Biochemistry 17, 6-40, 1982.

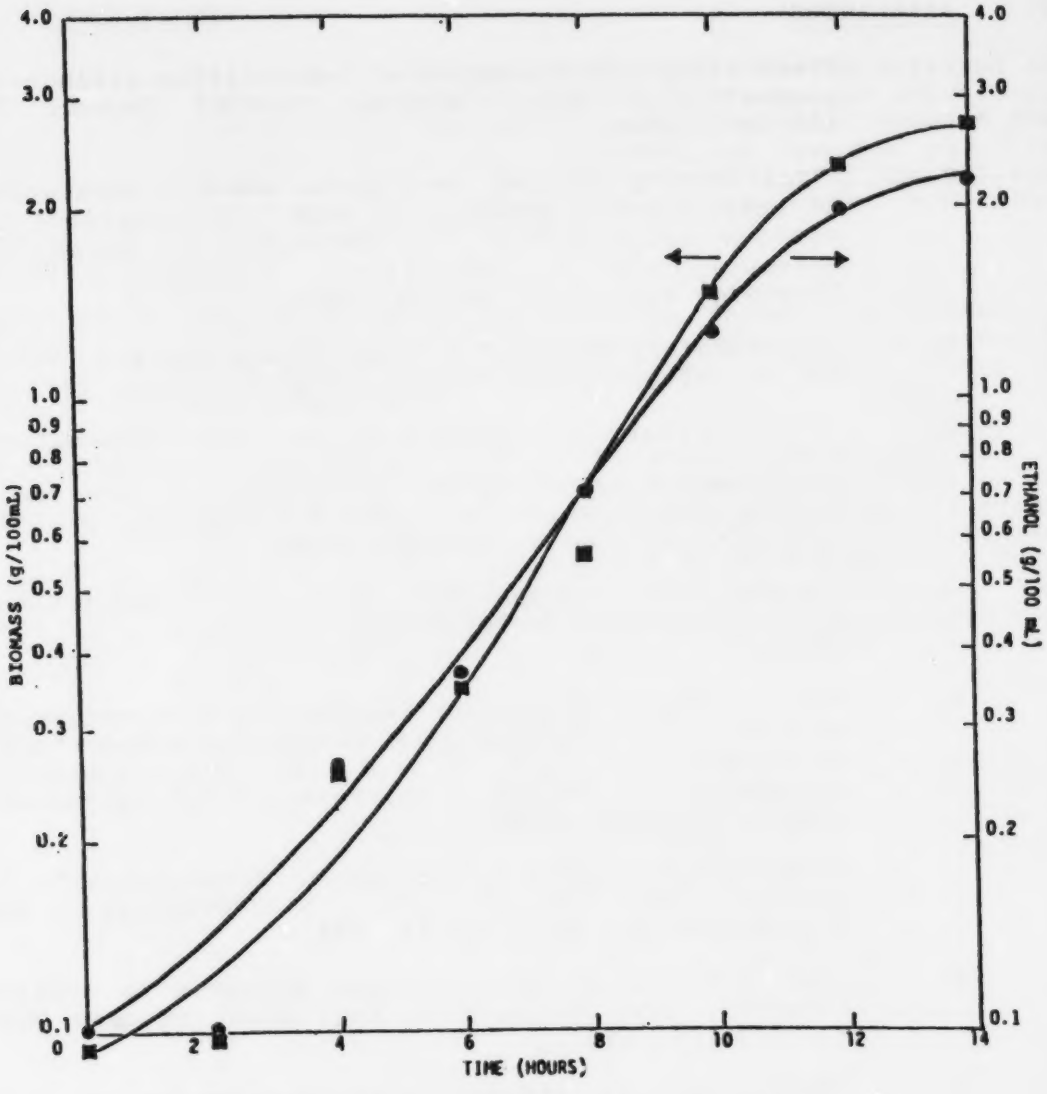


Figure 1 - Saccharomyces cerevisiae batch fermentation.

Table 1. Distribution coefficients and separation factors of ethanol in water and organic solvents

	Solvent	$D_{Ethanol}$	D_{Water}	S.F.	$(Solvent)_{Aq}$ mg/L	Remarks
1.	CCl_3F	1.1×10^{-2}	5.51×10^{-5}	2.0×10^{2}	1330	Separation of 2 layers sharp
2.	$CF_3CH_2OCF_2CF_2H$	2.7×10^{-2}	9.74×10^{-4}	27.7	695	Thin frothy line between the two clear layers
3.	$CF_3CHClBr$	4.8×10^{-2}	1.01×10^{-3}	47.9	1280	As above
4.	$CHClFCF_2OCH_3$	1.03×10^{-3}	1.33×10^{-3}	7.74	1685	Layers clear, Organic layer yellowish
5.	$CHClFCClF_2$	6.06×10^{-2}	1.04×10^{-3}	58.2	1690	Clear separation
6.	$CHF_2CF_2OCH_3$	-	-	-	-	Very turbid, no separation of layers
7.	C_4F_{10}	Extractions too low to detect			<10	
8.	$CH_3CH_2CH_2CH_2F$*	4.2 *	-	-	-	Clear separation
9.	$CH_3(CH_2)_{11}OH$ 25°C -	3.4×10^{-1}	1.2×10^{-2}	28.3	45.6	Layers remained slightly turbid
	35°C -	2.2×10^{-1}	1.1×10^{-2}	20.0	75.4	Clear separation

* This is an estimated value. Alcohol in water was 2.5% and it is assumed that remaining alochol was in organic phase. The peaks of Fluorobutane and alcohol could not be separated with G.C.

Table 2. Effect of various extractants on the growth and alcohol formation by S cerevisiae

Extractants		Biomass		Alcohol Conc. %	Glucose Conc. %
Name	Conc %	Growth mg/L	% Inhibition		
1. CCl_3F	0	5065	-	4.0	< 0.07
	0.5	2029	59.9	0.43	10.48
	1.0	2080	58.9	0.41	10.64
	2.0	2061	59.3	0.43	10.50
	3.0	2296	54.7	0.43	12.49
	5.0	2378	53.1	0.49	12.08
	10.0	2655	47.6	0.64	12.64
2. $CF_3CH_2OCF_2CF_2H$	0	4146	-	1.84	Traces
	0.1	4035	3	1.92	"
	0.2	3654	12	1.82	"
	1.0	2954	29	2.28	"
	2.0	2907	30	1.80	"
	5.0	2727	34	1.64	"
3. $CF_3CHClBr$	0	1054	-	2.1	6.1
	0.5	411	61	0.4	10.1
	1.0	461	56	0.5	8.5
	2.0	104	90	0.4	9.65
	3.0	120	89	0.4	9.1
	5.0	37	97	0.4	8.75
	10.0	0	100	0.4	8.6
4. $CHClFCF_2OCH_3$	0	3506	-	2.4	6.3
	0.5	301	91	Traces	8.8
	1.0	254	93	"	8.9
	2.0	251	93	"	9.5
	3.0	143	96	"	8.9
	5.0	96	97	"	9.8
	10.0	0	100	"	9.3
5. $CHClFCClF_2$ (Arcton 123A)	0	6200	-	2.8	3.4
	1.0	260	95.8	0.5	8.9
	2.0	260	95.8	0.4	9.5
	5.0	230	96.3	0.3	9.5
	10.0	130	97.9	0.4	9.3
6. $CHF_2CF_2OCH_3$	0	3710	-	3.0	2.5
	0.1	2669	28	1.22	6.6
	0.2	1902	49	0.24	6.3
	0.5	1380	63	0.69	5.64
	1.0	930	75	0.21	7.00
	2.0	0	100	0.14	7.68
	5.0	0	100	-	-

Cont'd ...

Table 2. Effect of various extractions on the growth and alcohol formation by S cerevisiae

Extractants		Biomass		Alcohol Conc. %	Glucose Conc. %
Name	Conc %	Growth mg/L	% Inhibition		
7. C_6F_{14}	0	3600	-	-	-
	1.0	3700	0	-	-
	2.0	-	-	-	-
	5.0	3650	0	-	-
	10.0	3700	0	-	-
8. $CH_3CH_2CH_2CH_2F$	0	968	-	1.20	6.3
	0.1	283	71	0.37	7.99
	0.2	14	99	0.36	8.05
	0.5	76	92	0.4	8.84
	1.0	0	100	0.4	8.80
	2.0	-	-	0.36	9.16
	5.0	0	100	0.4	8.88
9. $CH_3(CH_2)_{11}OH$	0	5278	0	4.7	Traces
	0.2	1706	68	0.8	7.66
	0.5	1719	67	1.3	8.00
	1.0	1729	67	1.2	8.40
	2.0	1519	71	0.7	10.24
	5.0	2128	60	0.8	8.56
	10.0	1706	68	0.7	9.52

Table 3. Effect of various extractants on the growth and alcohol formation by Z. mobilis

Extractants			Biomass			
	Name	Conc. %	Conc. mg/L	% Reduction	Alcohol Conc.	Glucose Conc.
1.	$BClCHCF_3$	0	80		Traces	2.52
		0.1	71	11.1	"	3.52
		0.2	0	100	"	2.72
		0.5	0	100	"	2.38
		1.0	0	100	"	2.72
		2.0	0	100	"	2.38
		5.0	0	100	"	2.8
2.	$CF_3HCF_2OCH_3$	0	80	-	Traces	2.52
		0.1	0	100	"	2.3
		0.2	0	100	"	2.04
		0.5	0	100	"	2.86
		1.0	0	100	"	2.86
		2.0	0	100	"	2.86
		5.0	0	100	"	2.86

Table 4. Comparison of the two continuous flow fermenters

Sampling Date	Fermenter #1 (Control)			Fermenter #2 (with 5g/day CCl_3F)		
	Biomass mg/L	Glucose Conc. %	Alcohol Conc. %	Biomass mg/L	Glucose Conc.	Alcohol Conc.
21/12/83	2697	1.4	3.04	1446	1.6	2.72
23/12/84	2378	-	-	1699	-	-
2/1/84	2280	0.46	3.64	1624	0.6	3.16
5/1/84	2507	-	-	1668	-	-
9/1/84	2172	0.85	3.25	1139	1.5	2.95
12/1/84	2490	-	-	1368	-	-
16/1/84	1972	-	-	1303	-	-
19/1/84	2682	1.4	3.04	2064	4.2	2.17

Table 5. The volume recovered at various temperatures during the distillation of 10% alcohol solution in Fluorocarbon 11.

Volume Collected ml.		Temperature °C
Start of distillation		23
10		25
20		25
30		28
40		28
50		28
60		29
70		98
Final distillation		98
Residue	0.5 ml	
Loss	23.5 ml	

Table 6. Effect of Fluorocarbon II on growth and alcohol fermentation

	Freon Conc. %	Biomass mg/L	Inhibition %	Alcohol Conc. %	Glucose Conc. %
1.	0	5065	-	4.0	< 0.07
	0.5	2029	59.9	0.43	10.48
	1.0	2080	58.9	0.41	10.64
	2.0	2061	59.3	0.43	10.50
	3.0	2296	54.7	0.43	12.49
	5.0	2378	53.1	0.49	12.08
	10.0	2655	47.6	0.64	12.64
2.	0	3506	-	3.41	0
	0.5	446	87.3	0.28	10.5
	1.0	350	90.0	0.30	10.2
	2.0	304	91.3	-	-
	3.0	302	91.4	0.3	-
	5.0	270	92.3	0.24	10.72
	10.0	205	94.2	0.2	9.8
3.	0	3780	-	3.55	0
	0.5	1888	50.1	1.26	6.4
	1.0	223	94.1	0.2	11.0
	2.0	224	94.1	-	-
	3.0	330	91.3	0.2	-
	5.0	250	93.4	0.2	11.2
	10.0	613	83.8	0.2	11.1